Biosafety Level 4

These questions are based on the Biosafety Level 4 section of *Biosafety in Microbiological and Biomedical Laboratories,* 3rd ed., pages 32-42.

Please circle the response that best describes the laboratory in which work with select agents will be carried out.

N.A. = not applicable. If you mark "N.A." , please provide a brief explanation below that item or on a separate page.

Standard Microbiological Practices

Yes, No, N.A. 1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

Yes, No, N.A. 2. Persons wash their hands after handling infectious materials and animals; they take a decontaminating shower when they leave the laboratory.

Yes, No, N.A. 3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

Yes, No, N.A. 4. Mouth pipetting is prohibited; only mechanical pipetting devices are used.

Yes, No, N.A. 5. All procedures are performed carefully to minimize the creation of aerosols.

Yes, No, N.A. 6. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Yes, No, N.A. 7. An insect and rodent control program is in effect.

Special Practices

Yes, No, N.A. 1. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Therefore, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as children or pregnant women, are not allowed in the laboratory or animal rooms.

Yes, No, N.A. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazards control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for insuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook, signed by all personnel, indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

Yes, No, N.A. 2. When infectious materials or infected animals are present in the laboratory or animal rooms, hazard warning signs, incorporating the universal biohazard symbol, are posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

Yes, No, N.A. 3. The laboratory director is responsible for insuring that, before working with organisms at Biosafety Level 4, all personnel demonstrate a high proficiency in standard microbiological practices and techniques, and in the special practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in these unique safe microbiological practices and techniques.

Yes, No, N.A. 4. Laboratory personnel receive available immunizations for the agents handled or potentially present in the

laboratory.

Yes, No, N.A. 5. Baseline serum samples for all laboratory and other at risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.

Yes, No, N.A. 6. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

Yes, No, N.A. 7. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural changes.

Yes, No, N.A. 8. Personnel enter and leave the facility only through the clothing change and shower rooms, and shower each time they leave the facility. Personnel use the airlocks to enter or leave the laboratory only in an emergency.

Yes, No, N.A. 9. Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including under garments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the facility. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing in the inner change room. Soiled clothing is autoclaved before laundering.

Yes, No, N.A. 10. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

Yes, No, N.A. 11. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

Yes, No, N.A. a. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

Yes, No, N.A. b. Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.

Yes, No, N.A. c. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, according to any local, state, or federal regulations.

Yes, No, N.A. 12. Biological materials to be removed from the Class III cabinet or from the Biosafety Level 4 laboratory in a viable or intact state are transferred to a nonbreakable, sealed primary container and then enclosed in a nonbreakable, sealed secondary container. This is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

Yes, No, N.A. 13. No materials, except for biological materials that are to remain in a viable or intact state, are removed from the Biosafety Level 4 laboratory unless they have been autoclaved or decontaminated before they leave the facility. Equipment or material which might be damaged by high temperatures or steam may be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Yes, No, N.A. 14. Laboratory equipment is decontaminated routinely after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Contaminated equipment is also decontaminated before it is sent for repair or maintenance.

Yes, No, N.A. 15. Spills of infectious materials are contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with concentrated infectious material.

Yes, No, N.A. 16. A system is set up for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for the quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

Yes, No, N.A. 17. Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

Safety Equipment (Primary Barriers)

Yes, No, N.A. 1. All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system.

Activities with viral agents that require Biosafety Level 4 secondary containment capabilities can be conducted within Class II biological safety cabinets within the facility, without the one-piece positive pressure personnel suit being used if (a) the facility has been decontaminated, (b) no work is being conducted in the facility with other agents assigned to Biosafety Level 4, (c) all personnel are immunized against the specific agent being manipulated and demonstrate protective antibody levels, and (d) all other standard and special practices are followed.

Yes, No, N.A. 2. All personnel entering the facility will don complete laboratory clothing, including undergarments, pants, and shirts or jumpsuits, shoes, and gloves. All such personal protective equipment is removed in the change room before showering and leaving the laboratory.

Laboratory Facility (Secondary Barriers)

Yes, No, N.A. 1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Yes, No, N.A. 2. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other ventilation lines contain HEPA filters.

Yes, No, N.A. 3. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

Yes, No, N.A. 4. Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Yes, No, N.A. 5. Laboratory furniture is of simple and sturdy construction, and spaces between benches, cabinets, and equipment are accessible for cleaning.

Yes, No, N.A. 6. A foot, elbow, or automatically operated hand washing sink is provided near the door of each laboratory room in the facility.

Yes, No, N.A. 7. If there is a central vacuum system, it does not serve areas outside the facility. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent backflow.

Yes, No, N.A. 8. If water fountains are provided, they are foot operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the backflow-protected distribution system supplying water to the laboratory areas.

Yes, No, N.A. 9. Access doors to the laboratory are self-closing and lockable.

Yes, No, N.A. 10. Any windows are breakage resistant.

Yes, No, N.A. 11. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall, and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

Yes, No, N.A. 12. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

Yes, No, N.A. 13. Liquid effluents from laboratory sinks, biological safety cabinets, floor drains (if used), and autoclave chambers are decontaminated by heat treatment before being discharged to the sanitary sewer. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically by use of a constant recording temperature sensor in conjunction with an indicator microorganism having a defined heat susceptibility profile.

Yes, No, N.A. 14. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to assure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate malfunction of the system. The airflow in the supply and exhaust components is monitored and the components interlocked to assure inward (or zero) airflow is maintained.

Yes, No, N.A. 15. The general room exhaust air from a facility in which the work is conducted in a Class III cabinet system is treated by a passage through a HEPA filter(s) prior to discharge to the outside. The air is discharged away from occupied spaces and air intakes. The HEPA filter(s) are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. The HEPA filter housings are designed to allow for *in situ* decontamination of the filter prior to removal, or removal of the filter in a sealed gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service-life of the exhaust HEPA filters can be extended through adequate filtration of the supply air.

Yes, No, N.A. 16. A specially designed suit area may be provided in the facility to provide personnel protection equivalent to that provided by Class III cabinets. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life support system. The life support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. The exhaust air from the suit area is filtered by two sets of HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit area.

Yes, No, N.A. 17. The treated exhaust air from Class II biological safety cabinets, located in a facility in which workers wear a positive pressure suit, may be discharged into the animal room environment or to the outside through the facility air exhaust system. The biological safety cabinets are tested and certified at 12-month intervals. The air exhausted from Class III biological safety cabinets is passaged through two HEPA filter systems (in series) prior to discharge to the outside. If the treated exhaust is discharged to the outside through the facility exhaust system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust system.